Final synopsis of Ph.D. thesis entitled

INFLUENCE OF NOBLE METAL ON NANO FERRITES: SYNTHESIS, CHARACTERIZATION AND APPLICATION

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CONTENTS

Particulars	Page No.
Objectives	1
Statement of the Research Problem	1
Nature and Scope of the Work Done	1
Research Methodology	2
Contribution Made to the Existing Knowledge	3
Organization of Thesis	3
Conclusion	8
References	9
List of Publications/Conferences Attended	10

1. Objectives

The objectives of the research work entitled "***Influence of noble metal on nano ferrites: Synthesis, Characterization and Application***" include:

a) To utilize plant extract as biofuel for greener synthesis
b) To synthesize novel nanomaterials with noble metal substituted cobalt ferrite
c) To investigate the effect of noble metal concentration on the morphology of the cobalt ferrite
d) To characterize the structural properties using various analytical techniques
e) To analyse magnetic, optical, electrical, dielectric properties
f) To investigate the antibacterial, antifungal, antibiofilm, antioxidant, anticancer and hyperthermia properties
g) To compare the properties of ferrites with and without noble metal substitution to understand the unique contributions of noble metal addition

2. Statement of the Research Problem

The development and optimization of ferrite materials by green synthesis have garnered significant interest due to their wide range of applications in electronics, magnetics, biomedical, and catalysis. However, the intrinsic properties of conventional ferrites often limit their performance in advanced technological applications. Doping ferrites with various elements as well green synthesis approach have been a common strategy to enhance their properties, but the influence of specific dopants, such as noble metal, remains inadequately explored. The incorporation of noble metal into ferrite structures promises to impart unique properties, such as enhanced biomedical, optical, electrical conductivity, improved magnetic behaviour, and superior catalytic activity. Despite these potential advantages, a systematic and comprehensive study is lacking in the current scientific literature. The primary research problem addressed in this study is to investigate the impact of noble metal substitution on the structural, magnetic, optical, electrical, biomedical properties of cobalt ferrite. By addressing this research problem, the study aims to fill the knowledge gap in the field of metal substituted ferrites, providing valuable insights into the development of high-performance materials for advanced technological applications.

3. Nature and Scope of the Work Done

Literature review: Ferrite is a fascinating magnetic material due to its unique properties and applications in biomedical, organic synthesis, optical, magnetic, and electronics. They exhibit ferrimagnetic properties, making them crucial in various technological applications. Traditional ferrites, however, often face limitations in performance, driving the exploration of doped ferrites to enhance their properties. Doping ferrites with different elements is a common strategy to tailor their

magnetic, electrical, and structural properties. Elements like cobalt, zinc, and rare-earth metals have been extensively studied for their effects on ferrite properties. Multifunctional nanomaterials are entities that combine the remarkable qualities of two or more nanomaterials. Incorporation of noble metal in ferrite is a relatively new and less explored area compared to other dopants. Noble metals are known for their high electrical conductivity, antimicrobial properties, and ability to enhance catalytic activity. Substitution of noble metal into ferrite structures could potentially lead to materials with unique and improved properties, suitable for advanced applications. Appropriate noble metal doping in suitable ferrite represents a promising avenue for enhancing material properties and expanding application potential. Hence, further research is needed to optimize synthesis methods in terms of green chemistry principals, thoroughly characterize the properties, and applications. This will pave the way for the practical implementation of these advanced materials in various technological fields.

Present work: The current research focuses on the systematic study of two well-known noble metals such as silver (Ag) or ruthenium (Ru) substitution in cobalt ferrite, aiming to understand the impact on the structural, magnetic, optical, electrical, elastic, and biomedical properties of cobalt ferrite. The work is organized into three main phases: synthesis, characterization, and application. The current research work begins with selection of appropriate noble metal and plant exact based on existing knowledge. The multifunctional nanomaterials were designed, by merging different properties of noble metal and cobalt ferrite through substitution of noble metal to iron (Fe) within a single framework. The aqueous *Moringa oleifera* gum extract was utilized as bio fuel in sol gel synthetic method. The synthesized nanomaterials were well characterized and subjected to comprehensive physical and biological evaluation to assess their magnetic, optical, elastic, electric, dielectric, hyperthermia, antibacterial, antifungal, antibiofilm, antioxidant, and anticancer activities.

Overall, the nature and scope of the work encompassed in the title involve comprehensive synergetic effect to develop a deep understanding of noble metal substituted cobalt ferrites and pave the way for their practical application in advanced technological fields.

4. Research Methodology

Various research methodologies utilized to achieve the objectives of the research work are;

a) Utilized scientific search engines like SciFinder and databases like PubMed, Scopus, Lens Patent Search, Google Patents, and Google Scholar to collect/ analyze relevant research articles, reviews, and patents
b) Screened and selected green reagent in classical and advanced methods for the synthesis

c) Characterization with techniques such as X-ray diffraction (XRD), UV–visible spectroscopy (UV), Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDX), transmission electron microscopy (TEM)
d) Evaluation of physical properties like magnetic using vibrating sample magnetometry (VSM); optical and photoluminescence (PL) properties using UV spectroscopy; elastic by using the ultrasonic pulse transmission technique; electrical using impedance spectroscopy technique, dielectric properties
e) Assessment of biological activities like anti-cancer activity and biocompatibility by using 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay; apoptotic efficacy by using high content imaging (HCI) assay; antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay; antibacterial, antibiofilm, and antifungal activity by minimal inhibitory concentration (MIC) determination using standard agar dilution method; cell morphology analysis by using TEM; efficiency of ferrite to absorb the energy of an alternating magnetic field and convert it into heat by using hyperthermia study

5. Contribution Made to the Existing Knowledge

The present research on the influence of either silver or ruthenium on properties of cobalt ferrite make significant contributions to the existing body of knowledge in several key areas. Importance of green synthesis, advantage of utilized plant extract as biofuel, enhanced physical and biological activities offer stepping stone in biomedical applications to accelerate drug discovery and development. This research significantly advances the understanding the synergetic effect of noble metal and ferrites, offering a valuable educational and scientific resource as new knowledge to researchers world-wide. This can be built upon by the scientific community and industry to develop high-performance magnetic materials for a wide range of technological applications to foster collaboration and inspiring further research in nanomaterial and nanomedicine.

6. Organization of Thesis

The present research work carried out in my research tenure has been combined in the form of a thesis entitled "***Influence of noble metal on nano ferrites: Synthesis, Characterization and Application***." This work has been broadly divided into five chapters.

Chapter 1: Introduction and Literature Survey

Considering the unique properties of cobalt ferrite ($CoFe_2O_4$) nanoparticles [1,2] like ease of synthesis, large magneto crystalline anisotropy, high saturation magnetization (MS), coercivity (HC), high Curie temperature, narrow band gap, excellent permeability, high chemical/electrochemical stability, and mechanical hardness. It is the second most studied

magnetic material, with a versatile application in biomedical and industrial fields. [3] Doping with noble metal cations is the most significant option for boosting the application of $CoFe_2O_4$ in the biomedical field. [4] Silver, gold, palladium, platinum, ruthenium, rhodium, iridium, and rhenium are examples of noble metals. Noble metals, which exhibit exceptional corrosion resistance and high temperature stability, have been successfully used as therapeutic agents in medicine since ancient times. Nanometric noble metals exhibit several uses, especially in biomedicine, where they have the potential to revolutionize many diseases are detected and treated. [4]

Specifically, by adding noble metals and iron oxides to afford diversified nanostructures with enhanced properties. [5] Nanomaterials offer a promising avenue for antimicrobial and anti-cancer therapies by leveraging structural benefits such as intrinsic composition, morphology, dimensions, and surface properties. Utilizing the heat generated by nano-heaters like magnetic nanoparticles (ferrite) under an alternating magnetic field, magnetic hyperthermia (MHT) emerges as a promising approach for cancer treatment, as it selectively damages tumor tissue. [6] Comparable to all the synthesis methods available, the sol-gel technique stands out as a convenient approach due to its straightforward preparation process, cost-effectiveness, ability to achieve precise control over size and morphology, and uniform phase distribution, particularly in complex systems such as ferrites [7]. However, many traditional approaches are costly and environmentally harmful. Furthermore, natural resources such as plant extracts, have proven to be highly effective in the eco-friendly green synthesis of nano cobalt ferrite [8].

Magnetic hyperthermia therapy (MHT) can serve as either an independent treatment or a supplementary therapy to enhance the effectiveness of radiation and chemotherapy.[9] *Moringa oleifera* is a medicinal plant from the *Moringaceae* family. It is popularly known as the drumstick tree or the miracle tree in India. The various sections of *Moringa* were successfully used for the green synthesis of metal and metal oxide nanomaterials. Recently, *M. oleifera* leaf extract and gum were successfully utilized for the synthesis of silver nanoparticles (AgNPs). [10]

<u>Chapter 2</u>: Synthesis of $CoM_xFe_{2-x}O_4$ (M= Ag, Ru) using *Moringa oleifera* gum extract

Aqueous *Moringa oleifera* gum extract, serves as an excellent natural chelating and reducing agent. This method aligns with green chemistry principles, reducing the need for hazardous chemicals and providing a sustainable alternative for synthesizing cobalt ferrite. This method not only minimizes the use of hazardous chemicals but also simplifies the synthesis process. *Chemicals and Reagents:* The analytical-grade reagents were purchased from Sigma-Aldrich: ferric nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$), ruthenium (III) chloride trihydrate [$RuCl_3 \cdot 3H_2O$], silver nitrate ($AgNO_3$), *M. oleifera* gum, deionized water, and ammonia solution (NH_4OH).

Figure 1. *M. oleifera* gum -assisted synthesis of $CoAg_xFe_{2-x}O_4$.

Preparation of Noble metal Substituted nano cobalt ferrite: Nobel metal-substituted cobalt ferrite nanohybrids with the composition of $CoM_xFe_{2-x}O_4$ (M= Ag, Ru; x = 0, 0.1, 0.2, 0.3, 0.4) were synthesized by the sol–gel method, as shown in Figure 1. The compound was prepared by mixing a stoichiometric ratio of ferric nitrate, cobalt nitrate or ruthenium chloride, and silver nitrate and dissolving it in deionized water. Aqueous *M. oleifera* gum extract was added in a molar ratio of 1:1. The pH of the solution was adjusted to 7 by dropwise addition of ammonia solution under stirring. The obtained solution was placed on a hot plate at 80 °C to afford a thick viscous gel to furnish a ferrite powder. The obtained powder was crushed to a fine powder with an agate mortar and subjected to calcination at 600 °C for 5 h in a temperature-controlled furnace.

Chapter 3: Experimental techniques to characterization of $CoM_xFe_{2-x}O_4$ (M= Ag, Ru)

Characterizing noble metal substituted cobalt ferrite is essential to understanding its structural, magnetic, electrical, and biomedical properties. The key experimental techniques used for comprehensive characterization:

1. XRD: To determine the phase composition, crystallite size, and crystal structure of the synthesized material. XRD pattern with peaks corresponding to different crystal planes, phase identification, and crystallite size.
2. SEM: To examine the surface morphology and particle size of the synthesized ferrite. High-resolution images showing the surface structure, particle size, and morphology.
3. TEM: To investigate the internal structure, particle size, and distribution of elements at a higher resolution. Detailed images of the internal structure, particle size, and SAED patterns for crystal structure analysis; cell morphology analysis
4. EDX: To confirm the presence and distribution of elements, including silver, in the ferrite. EDX spectra showing the elemental composition and mapping images showing the distribution of elements.
5. VSM: To measure the magnetic properties, such as saturation magnetization, coercivity, and magnetic hysteresis. Magnetic hysteresis loops providing data on saturation magnetization, coercivity, and remanence.
6. Dielectric Measurements: To evaluate the dielectric constant and dielectric loss of the material. Frequency-dependent plots of dielectric constant and dielectric loss.

7. FTIR: To identify the functional groups and chemical bonds present in the material. FTIR spectra showing absorption bands corresponding to different functional groups and chemical bonds.
8. UV spectroscopy: optical and photoluminescence (PL) properties
9. Ultrasonic pulse transmission technique: elastic properties
10. Impedance spectroscopy technique: electrical properties
11. Dielectric properties
12. MTT assay: anti-cancer activity, cytotoxicity, and biocompatibility
13. HCI assay: apoptotic efficacy
14. DPPH assay: antioxidant activity
15. Standard agar dilution method: minimal inhibitory concentration (MIC) determination for antibacterial, antibiofilm, and antifungal activity
16. Hyperthermia study: The combined use of these experimental techniques provides a comprehensive understanding and this detailed characterization is crucial for optimizing the synthesis process and tailoring the material for specific applications in biomedicine.

Chapter 4: Effect of silver on biological applications of cobalt ferrite

The sol–gel method was employed to prepare nano $CoFe_2O_4$ and silver-substituted $CoFe_2O_4$ nanohybrids ($CoAg_xFe_{2-x}O_4$, x = 0, 0.1, 0.2, 0.3, 0.4) utilizing *Moringa oleifera* gum as biofuel. The morphology, size, shape, magnetic, optical, and functional groups of the crystallites were determined using various techniques such as UV–visible, Fourier transform infrared, X-ray diffraction, Rietveld, scanning electron microscopy, transmission electron microscopy, vibrating sample magnetometry, photoluminescence, SEM-EDX, and Raman spectroscopy. The optical properties were investigated in two different bands in the photoluminescence emission spectra at 469 and 493 nm. Dielectric properties were examined across different frequencies, and their dispersion was analyzed using Koop's theory in alignment with Maxwell-Wagner polarization. Furthermore, antibacterial (Gram-positive bacteria bacterial strains, *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative bacterial strains, *Pseudomonas aeruginosa*, and *Escherichia coli*), antibiofilm activity (*E. coli*), and antioxidant (DPPH) activities were investigated.

The produced nanoferrite has a spherical shape with cubic spinal structures. The findings suggest that as the Ag content increases, there is an augmentation in the lattice parameter values. Saturation magnetization (Ms) and coercivity (Hc) decrease as the Ag content increases significantly. The Raman spectra of cobalt ferrite exhibited incremental alterations with the rise in Ag substitution. Elastic properties revealed a decline in elastic constants with higher Ag content.

Notably, Ag substitution displayed a direct correlation between average sound velocity and elastic constants. Cole-Cole plots of investigated samples showed single semicircle which indicates that the electrical conduction is due to grain boundaries.

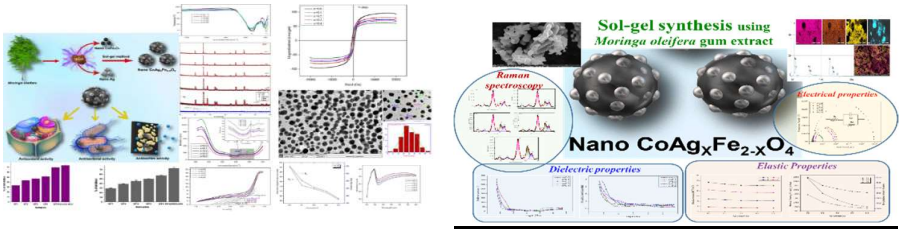

Figure 2. Synthesis of $CoAg_xFe_{2-x}O_4$ with characterization and applications

It is specified that the lattice constant increases with an increase in the concentration of Ag. The substantial increase in the silver content offers a constructive impact on optical properties. The VSM investigation indicates that coercivity (Hc) and saturation magnetization (Ms) decline with an increase in the silver percentage. The enhanced antimicrobial, antibiofilm, and antioxidative properties were predominantly attained by augmenting surface reactivity via appropriate surface area and nanoscale dimensions. It became apparent that the quantity of silver in cobalt ferrite subsequently enhanced the antibacterial properties. The substantial increase in the silver content offers a constructive impact on studied biomedical activities. The synthesized nanoparticles demonstrate enhanced hyperthermic capabilities when exposed to an alternating magnetic field, offering a promising avenue for cancer treatment. The results suggest that the synergistic combination of silver in cobalt ferrite nanoparticles holds significant promise for advancing hyperthermia-based cancer therapies. Hence, offer new insights into the development of effective and targeted treatments for cancer and the evolving landscape of nanomedicine.

<u>Chapter 5</u>: Effect of ruthenium on biological applications of cobalt ferrite

$CoRu_xFe_{2-x}O_4$ were synthesized by sol-gel method using *Moringa oleifera* gum as biofuel. The study explores the unique structural and magnetic properties imparted by the incorporation Ru ions into the cobalt ferrite lattice. *In vitro* MTT assay revealed the selective cytotoxicity of the nanohybrids against breast KAIMRC2, MDAMB231 and colorectal, HCT8, HCT116 cancer cells lines. High content imaging complimented MTT results. TEM analysis showcased significant morphological alterations by the intracellular localization of nano-ferrites on KAIMRC-2 cell morphology. The synthesized nanoparticles demonstrate enhanced hyperthermic capabilities when exposed to an alternating magnetic field, offering a promising avenue for cancer treatment. The results suggest that the synergistic combination of ruthenium in cobalt ferrite nanoparticles holds significant promise for advancing hyperthermia-based cancer therapies.

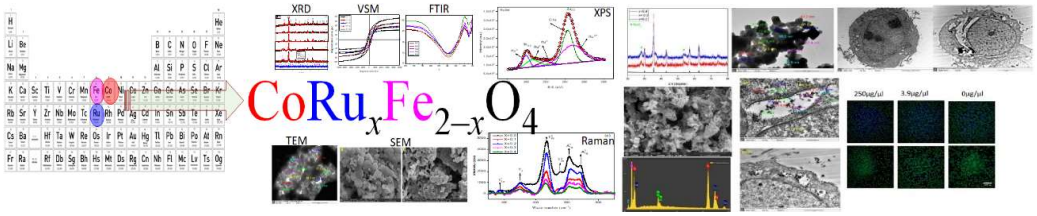

Figure 3. Synthesis, Characterization and applications of $CoRu_xFe_{2-x}O_4$

XRD peaks correspond to cubic spinel structures within the Fd3m space group. SEM images reveal that the synthesized nanohybrids display a spherical shape and tend to group together as a single big particle. This aggregation phenomenon is attributed to magnetic interactions between individual nanoparticles. The analyzed aggregated formations demonstrate an uneven dispersion of hybrids within the prepared samples. The absence of peaks corresponding to any other elements in Energy Dispersive X-ray (EDX) suggests that the nanoparticles consist solely of iron, cobalt, ruthenium, and oxygen, without any noticeable impurities or contaminants. This observation serves as a significant indication of the purity of the synthesized nanoparticles. TEM image revealed a mixture of irregular and spherical shapes among the nanoparticles, with the majority displaying a uniform morphology. All compounds exhibit biocompatibility at low concentration and cytotoxicity effect at higher doses. Apoptotic efficacy confirmed by using high content imaging (HCI) assay. Cell death was observed using TEM before and after addition of $CoRu_xFe_{2-x}O_4$. Hence, these findings offer new insights into the development of effective and targeted treatments for cancer and the evolving landscape of nanomedicine.

Chapter 6: Conclusion and future scope

The research focused on the synthesis, characterization, and application of noble metal (silver, ruthenium) substituted cobalt ferrite, a promising material for advanced technological applications. The research successfully demonstrated that addition of silver or ruthenium in cobalt ferrite can significantly enhance its properties, making it suitable for a wide range of advanced applications. The use of aqueous *Moringa oleifera* gum extract as a natural chelating and reducing agent offers an environmentally friendly approach for synthesis. It displays enhanced structural, magnetic, optical, electrical, elastic, and dielectric properties. Moreover, exhibit effective biomedical applications like antimicrobial, antioxidant, and hyperthermia treatment due to its biocompatibility for anti-cancer activities. This research lays a solid foundation for further exploration and practical implementation in advanced technological fields.

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9. List of Publications/Conferences Attended

1) Tamboli, Q.Y., Patange, S.M., Mohanta, Y.K., Sharma, R. and Zakde, K.R., **2023**. Green synthesis of cobalt ferrite nanoparticles: An emerging material for environmental and biomedical applications. *Journal of Nanomaterials*, 2023(1), p.9770212. https://doi.org/10.1155/2023/9770212
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9) "Eco friendly synthesis of Ag substituted $ZnFe_2O_4$ as magnetically isolable catalysts for acylation of alcohol" as ICMSN-SA2023/A-10 in *International conference on materials and nanotechnology for sustainable applications (ICMSNSA-2023)* at MGM University, Aurangabad, India on 23-24 March 2023.

10) "Electron microscopical investigation of the morphology and anti-cancer activity of nano $CoAg_xFe_{2-x}O_4$" in *The 3rd Middle East Conference on Electron Microscopy* at Crown Plaza, Bahrain on 14 December 2023. **Poster Presentation**

11) "Synthesis and characterization of $CoRu_xFe_{2-x}O_4$ with enhanced hyperthermic properties for cancer treatment" in *ACS-RSC Regional MENA Conference 2024* at Doha, Qatar on 3-5 November 2024. **Poster Presentation**

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